

CLAIMS

What is claimed is:

1. A packaging cell line capable of complementing recombinant adenovirus based on a serotype from subgroup B.
2. The packaging cell line of claim 1 wherein said serotype from subgroup B is adenovirus type 35.
3. The packaging cell line of claim 1 or 2, wherein said packaging cell line is derived from primary, diploid human cells, or derivatives thereof, said primary, diploid human cells or derivatives thereof having been transformed by adenovirus E1 coding sequences either operatively linked on one DNA molecule or located on two separate DNA molecules, said adenovirus E1 coding sequences being operatively linked to regulatory sequences enabling transcription and translation of encoded proteins.
4. The packaging cell line of claim 3 wherein the primary, diploid human cells, or derivatives thereof have been selected from the group consisting of primary human retinoblasts, primary human embryonic kidney cells and primary human amniocytes.
5. The packaging cell line of claim 3 or 4, wherein the primary, diploid human cells, or derivatives thereof have been transfected with an adenovirus E1A coding sequence to induce unlimited proliferation.
6. The packaging cell line of claim 5 wherein said packaging cell line further comprises an E1B coding sequence.
7. The packaging cell line of claim 3 or 4, wherein the primary, diploid human cells, or derivatives thereof have been transformed by expression of adenovirus E1 proteins of a subgroup other than subgroup C.
8. The packaging cell line of claim 7 wherein the subgroup other than subgroup C is subgroup B.

9. The packaging cell line of claim 8, wherein said adenovirus E1 proteins are derived from adenovirus type 35.

10. The packaging cell line of claim 3 or claim 4, wherein the primary, diploid human cells or derivatives thereof have been transformed with a chimeric adenovirus E1 construct comprising part of a first adenovirus E1 coding sequence of a first adenovirus serotype that enables efficient transformation of primary human cells or derivatives thereof; and part of a second adenovirus E1 coding sequence of a second adenovirus serotype, wherein said second adenovirus E1 coding sequence provides the serotype-specific adenovirus E1B function(s) that enable(s) efficient propagation of recombinant adenovirus E1-deleted viruses of said second adenovirus serotype.

11. The packaging cell line of claim 10 wherein said first adenovirus serotype is a subgroup C adenovirus and said second adenovirus serotype is a subgroup B adenovirus, more particular adenovirus type 35.

12. The packaging cell line of claim 10 wherein E1A coding sequence and at least part of the E1B-21K coding sequence are derived from a subgroup C adenovirus, and the E1B-55K coding sequence as far as not overlapping with the 21K coding sequence is derived from a subgroup B adenovirus.

13. The packaging cell line of claim 12 wherein said subgroup B adenovirus is adenovirus type 35.

14. The packaging cell line of claim 10 wherein all E1 coding sequences are derived from a subgroup C adenovirus, except for at least a part of the E1B-55K coding sequence that is necessary for serotype-specific complementation of an alternative adenovirus serotype, said E1B coding sequence being derived from said alternative adenovirus serotype.

15. The packaging cell line of claim 6, claim 10 or claim 14, wherein said packaging cell line comprises bovine adenovirus E1B-55K.

16. The packaging cell line of claim 15, wherein said complementing recombinant adenovirus is derived from a bovine adenovirus.

17. The packaging cell line of claim 3 or 4, wherein the primary, diploid human cells or derivatives thereof have been transformed by adenovirus E1 coding sequences located on two separate DNA molecules wherein the first DNA molecule carries at least part of the E1 coding sequences of the serotype enabling efficient transformation and the second DNA molecule carries at least part of the sequences necessary for serotype-specific complementation.

18. The packaging cell line of claim 4 wherein said derivative cells are PER.C6 (ECACC deposit number 96022940) which further comprise an Ad35-E1 region integrated into their genome, and wherein said Ad35-E1 region is present in a functional expression cassette.

19. The packaging cell line of claim 18 wherein said Ad35-E1 region does not contain sequences overlapping with sequences present in an associated recombinant viral vector.

20. The packaging cell line of claim 18 or claim 19, wherein said functional expression cassette comprises a heterologous promoter and a poly-adenylation signal functionally linked to said Ad35-E1 region, wherein said heterologous promoter is a human phosphoglycerate gene promoter (hPGK) and wherein said poly-adenylation signal is a hepatitis B virus poly-adenylation signal (HBV-pA).

21. The packaging cell line of claim 20 wherein said Ad35-E1 region comprises the coding regions of the E1A proteins and the E1B promoter sequence linked to E1B coding sequences up to and including the stop codon of the E1B 55K protein.

22. The packaging cell line of claim 20 wherein said Ad35-E1 region comprises nucleotide 468 up to and including nucleotide 3400 of the Ad35 wild-type sequence.

23. A cell line derived from PER.C6 (ECACC deposit number 96022940), which cell line comprises Ad35-E1B coding sequences.

24. The cell line of claim 23 wherein said Ad35-E1B coding sequences are driven by an E1B promoter and terminated by a heterologous poly-adenylation signal.

25. The cell line of claim 23, wherein said Ad35-E1B coding sequences are driven by a heterologous promoter.

26. The cell line of claim 25 wherein said Ad35-E1B coding sequences are driven by a hPGK promoter or an Elongation Factor-1a (EF-1a) promoter and terminated by a heterologous poly-adenylation signal.

27. The cell line of claim 24 or 26, wherein said heterologous poly-adenylation signal is a hepatitis B virus poly-adenylation signal (HBV-pA).

28. The cell line according to any one of claims 23-27, wherein said Ad35-E1B coding sequences comprise the coding sequences of the E1B 21K and the E1B 55K proteins located between nucleotides 1611 and 3400 of the wild-type Ad35 sequence.

29. The cell line of claim 28 wherein said Ad35-E1B coding sequences comprise nucleotides 1550 to and including nucleotide 3400 of the wild-type Ad35 sequence.

30. The cell line of claim 28 wherein said Ad35-E1B coding sequences comprise the coding sequences of the E1B-55K gene located between nucleotides 1916 and 3400 of the wild-type Ad35 sequence.

31. The cell line of any one of claims 23 through 27, wherein said Ad35-E1B coding sequences comprise the coding sequences of the E1B-55K gene located between nucleotides 1916 and 3400 of the wild-type Ad35 sequence.

32. The cell line of claim 31, lacking a functional coding sequence for E1B-21K.

33. The packaging cell line of any one of claims 1 through 22, or the cell line of any one of claims 23-32, further comprising a DNA encoding at least E4-orf6 of an adenovirus of subgroup B.

34. A process for complementing a recombinant adenovirus, said method comprising:

providing the packaging cell line of any one of claims 1 through 22 or the cell line of any one of claims 23 through 33, with said recombinant adenovirus; and

culturing said cell to allow for complementation.

35. The process according to claim 34, further comprising harvesting complemented recombinant adenovirus.

36. The process according to claim 34 or claim 35, wherein said recombinant adenovirus is derived from a subgroup B adenovirus.

37. The process according to claim 36, wherein said recombinant adenovirus is derived from adenovirus type 35.

38. A recombinant adenovirus produced by the process according to any one of claims 34 through 37.

39. The recombinant adenovirus of claim 38, further having a deletion of nucleic acid encoding at least one E1-region protein.

40. The recombinant adenovirus of claim 38 or claim 39, further comprising a deletion of nucleic acid encoding at least one E3-region protein and/or at least one E4-region protein.

41. Use of a recombinant adenovirus according to any one of claims 38-40, for the preparation of a medicament.

42. Use of a packaging cell line according to any one of claims 1-22, or a cell line according to any one of claims 23-33, for the complementation of a recombinant adenovirus.